

The Zooxanthellal Tubular System in the Giant Clam

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Giant clams (family Tridacnidae) are special in that they contain large numbers of symbiotic dinoflagellates, Symbiodinium sp., commonly called zooxanthellae which live in the clam's siphonal mantle (hypertrophied siphonal tissues) (1) and are important in its nutrition (2, 3). In 1946, Mansour (4) partially described a tubular system arising from the clam stomach, extending into the mantle and containing zooxanthellae. However, the eminent scientist Sir Maurice Yonge (5, 6) disputed its existence. Subsequently, Yonge's views appear to have suppressed further investigations of Mansour's observations. The zooxanthellae have been universally regarded as living in the hemal spaces of the mantle (2, 5, 7, 8, 9). This study, however, has confirmed the presence of the tubular system indicated by Mansour and has shown that the zooxanthellae live within a branched, tubular structure that has no direct connection with the hemolymph. The existence of this tubular system has important implications for our understanding of the symbiosis between tridacnids and their symbiotic algae.

During a study of the anatomy and histology of giant clams, numerous *Tridacna gigas*, from a few millimeters to 35 cm in shell length, were dissected. Tissues were fixed in 10% seawater formalin, processed by routine histological methods, including serial sectioning, and stained with hematoxylin and eosin (H & E). Additional stains included Masson's trichome for muscle tissue, periodic Acid-Schiff (PAS) and PAS/diastase for glycogen, starch, neutral mucopolysaccharides and glycoproteins, and an Alcian Blue stain to delineate the tertiary zooxanthellal tubes.

The zooxanthellal tubular system was found to commence as a single primary tube, originating from one of

the digestive diverticular ducts of the stomach (Figs. 1a, b). It passes dorsally and posteriorly between the digestive diverticula, and then between the crystalline style sac and the muscular wall that encloses the digestive and reproductive organs. Above the digestive organs, it divides into right and left tubes. These tubes pass close to the dorsal aspect of the muscular wall, then through this muscular wall and into the kidney parenchyma. Both tubes travel through the kidney in a more ventral direction, until they leave the kidney and enter the root of the middle etenidial suspensory ligament. The tubes continue to travel posteriorly, where they become embedded in the connective tissue sheath of the adductor muscle. While traversing the posterior portion of the adductor muscle, each tube gives off a branch which is embedded in the floor of the excurrent water chamber. Each primary tube associated with the zooxanthellae passes toward the end of the adductor muscle, and dorsally into the root of the siphonal mantle, before branching both anteriorly and posteriorly. One or more main branches run along inside the root of the siphonal mantle close to the circumpallial artery, the circumpallial vein, and the pallial nerves. Secondary zooxanthellal tubes branch into the upper levels of the inner fold of the siphonal mantle, where they terminate in convolutions of thin tertiary tubes with blind ends. These tertiary tubes contain the zooxanthellae. Other secondary branches form tertiary tubes in other organs, such as in the connective tissue surrounding the adductor muscle and in the connective tissues of the bulbus arteriosus of the heart, the pericardium, the etenidia, and the lateral mantle.

The primary zooxanthellal tubes (Fig. 2) have an epithelial lining of cuboidal to low columnar cells with long cilia and are surrounded by a thin zone of muscle fibers. The secondary tubes (Fig. 3) are thin-walled and

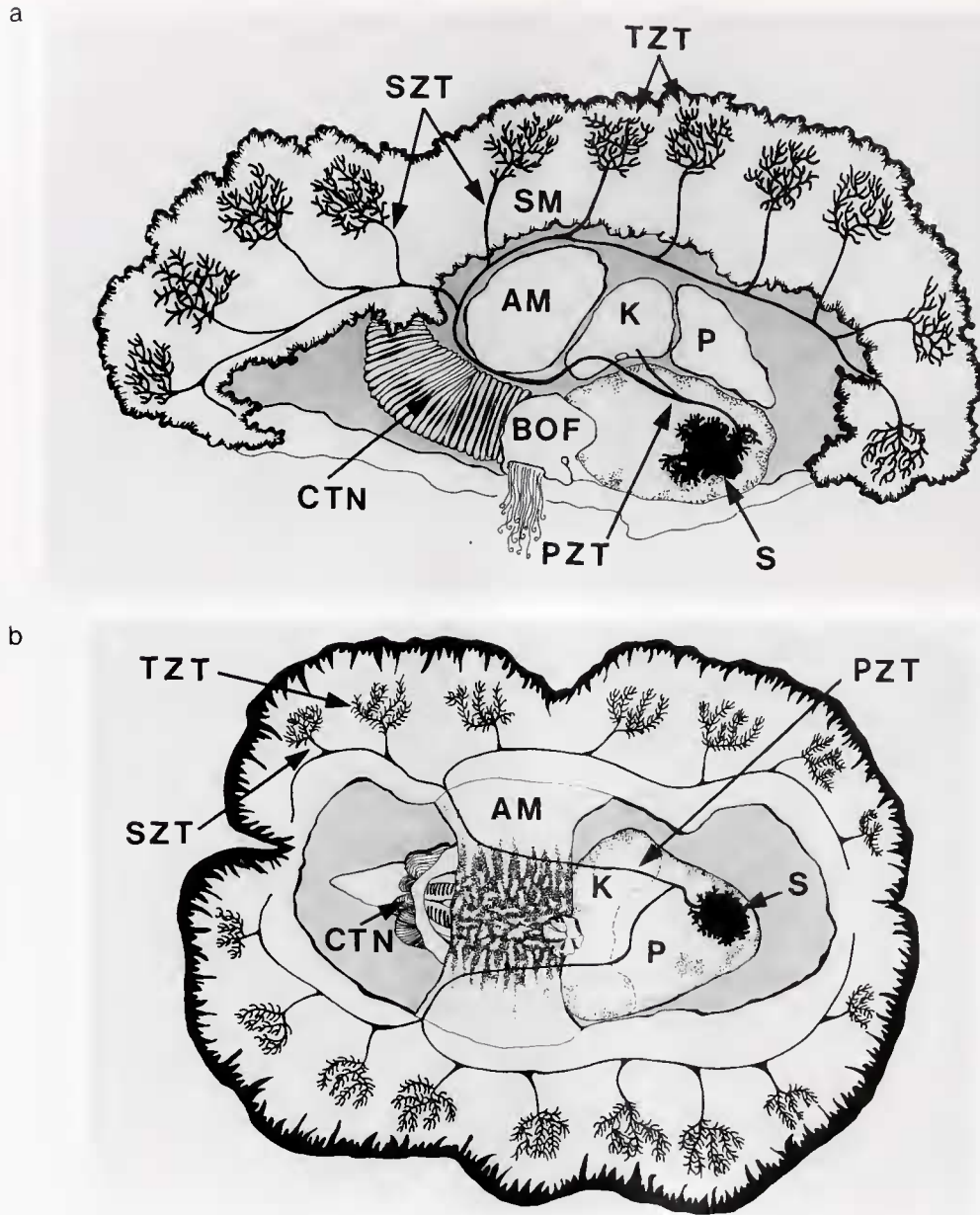


Figure 1. Diagrammatic view of the path followed by the zooxanthellal tube through a giant clam. The black, highly branched structure on the surface of the stomach (S) in both a and b is the mass of digestive diverticular. Note that the primary zooxanthellal tube (PZT) originates from one of the diverticular ducts. a: Medial view of a bisected clam. b: Dorsal view of a bisected clam.

Abbreviations: AM = adductor muscle; BOF = byssal organ/foot; CTN = ctenidia; K = kidney; P = pericardium; PZT = primary zooxanthellal tube; S = stomach; SM = siphonal muscle; SZT = secondary zooxanthellal tube; TZT = tertiary zooxanthellal tube.

are lined by ciliated epithelial cells with thin cytoplasm and small, prominent dark nuclei. Similar cells line the tertiary tubes (Fig. 3), but these cells appear to lack cilia. In naturally occurring bleached mantles, *i.e.*, in mantles lacking zooxanthellae, the tertiary zooxanthellal tubes atrophy.

No zooxanthellae were observed living free in the hemal sinuses, explaining previous reports that zooxanthellae are

not found in hemolymph samples from giant clams (10, 11). Although some elements of the tubular system associated with the zooxanthellae were partially described by Mansour (4), he did not recognize that the tertiary zooxanthellal tubes have blind ends and do not communicate with the hemolymph system. Parts of the tubular system have been described previously by others (2, 12), but interpreted as communicating with the hemolymphatic system.

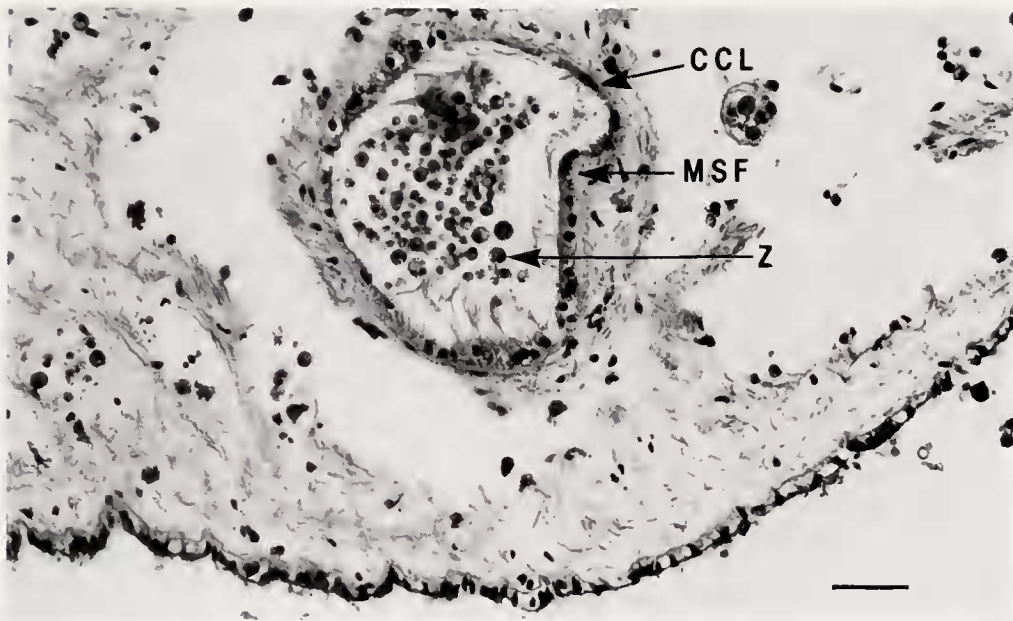


Figure 2. A primary zooxanthellal tube (transverse section, H & E stain. Bar = 19 μ m).
Abbreviations: CCL = ciliated columnar epithelium, MSF = muscle fibers; Z = zooxanthellae.

Our observations resolve much of the controversy that has existed over the past 50 years concerning the location and fate of zooxanthellae in the giant clams (2, 4, 5, 6, 7, 9, 10, 11, 13, 14, 15, 16, 17). The existence of this system simplifies the interpretation of previous data on the way

the symbiosis functions. For example, Yonge (16) drew attention to the need to regulate the numbers of zooxanthellae that reproduce in the siphonal mantle. Yonge hypothesized that the algae are culled from the siphonal mantle by amoebocytes, wherein they are digested, and

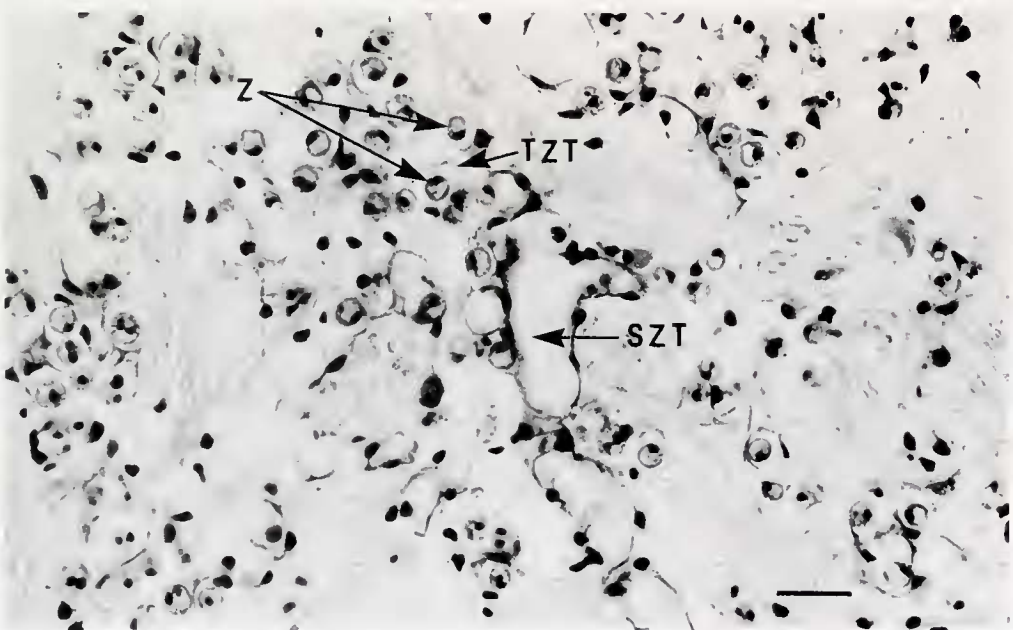


Figure 3. Secondary and tertiary zooxanthellal tubes in the siphonal mantle (transverse section, Alcian Blue stain. Bar = 23 μ m).
Abbreviations: SZT = secondary zooxanthellal tube; TZT = tertiary zooxanthellal tube, Z = zooxanthellae.

that the indigestible remains accumulate in the kidneys (18). As has been stated before, there is no evidence of this, and the algae move from the siphonal mantle to the stomach. Healthy zooxanthellae observed in the stomach (19), pass through the intestine and the rectum (2, 20) and are released in the feces; thus these algae, by an unknown mechanism, are able to resist host digestion. This route is also available for the mass expulsion of zooxanthellae from clams exposed to elevated environmental temperatures (21). Furthermore, there is no direct connection between the hemolymph and the stomach, via the tubes associated with the zooxanthellae, that would allow digestive enzymes into the hemolymphatic system (7). The entire branched tubular system associated with the zooxanthellae communicates with the stomach via a single opening, which is visible in clams that are only a few weeks old (22), and which would appear to explain the initial entry of zooxanthellae into the mantle (12).

The definition of this tubular system has implications for our understanding of the nutritional relationship between the giant clams and their zooxanthellae. It also reveals that the giant clam-zooxanthellae symbiosis is actually like other known invertebrate-algal symbioses, being intimately associated with the digestive system of the host (23).

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